General comments:

This manuscript addresses how to interface phytoplankton observations across many different lenses (e.g., metabarcoding, metatranscriptomics, HPLC, flow cytometry, biogeochemical rate measurements), a goal that has remained elusive due to differences in absolute quantification of organisms and relative abundances stemming from the compositional nature of molecular datasets. The authors circumvent this by using quantitative techniques, such as with the use of internal standards, to move beyond relative abundances with their molecular efforts. This allows them to complement other approaches like flow cytometry and HPLC used to measure pigment concentrations to reveal significant correlations between different eukaryotic and cyanobacteria phytoplankton groups across these different methodologies. By integrating the different approaches, they have further leveraged these relationships to interpret mechanisms setting the ecological patterns (e.g., productivity-diversity relationships, harmful algal bloom composition) in a dynamic upwelling region across both spatial and temporal dimensions.

Furthermore, since HPLC-measured pigments are routinely used to develop and validate remote sensing observation, including emerging high resolution hyperspectral remote sensing reflectance data, the authors highlight the importance of comparing phytoplankton pigments to alternate metrics, e.g., metabarcoding and metatranscriptomics, of phytoplankton community composition (PCC). The positive correlations between HPLC and molecular based PCC observed in this study are helpful in establishing the usefulness of using molecular data to further help validate global phytoplankton community structure being observed by remote sensing algorithms and developing improvements with Earth system models (ESMs).

In general, I find the authors did a nice job structuring the manuscript, building their arguments, and supporting their findings in context of what has been discussed in literature. The overall content and important take home messages are also clearly articulated. However, I think section 3.2.3 could use a bit more explicit discussion guiding how to interpret the results highlighted here and create a stronger link to how ESMs might use these results (or perhaps we should simply focus on the patterns observed as another validation reference for ESMs?).

Importantly, since so many of the relationships and ecological patterns discussed throughout the paper rely on various statistical analyses, I would strongly urge the authors to update the “Statistics” section in the methods and provide some justification for choosing Pearson correlation instead of Spearman correlations for this study (see more specific comments below for general guidelines that might be helpful). Lastly, there were several different sequencing platforms used for the various libraries prepared for metabarcoding and metatranscriptomics work – please address whether there are any biases or concerns comparing across all the different platforms (e.g., did you use unique

dual indexing pooling combinations to minimize index hopping with the NovaSeq 6000 platform).

Specific comments:

Figure 1: Panel D – I'm a little confused by the y-axis scale for nitrate concentrations. I think you are trying to highlight the often very low (<0.5 µM) concentrations on the same range as values as high as 20+ µM but the scaling seems a bit unorthodox. The intervals between values don't signify the same thing so is there a way to clarify that (perhaps in the figure legend)?

Methods

Section 2.4 & 2.5: It doesn't seem that any mock communities were used in the library prep, is that right? Please address how mock communities could also improve the quantitative assessment of this study (e.g., see conclusions from Lamb et al., 2018 - https://doi.org/10.1111/mec.14920).

For the use of Parada et al., 2016 primer set, were the 18S sequences discarded and solely the 16S sequences were denoised into ASVs? If yes, perhaps mention this – it seems to tally with your choice of removing all eukaryotic chloroplast and mitochondrial ASVs from the 16S fraction of this data (lines 206 – 207).

Lines 211 – 212: In previous method section (2.3), only the addition of *S. pombe* is mentioned so please reconcile that before introducing this step of dividing by ratio of an additional internal standard of *T. thermophilus*.

Section 2.7 Statistics: Please expand upon this section to highlight the different functions and any parameters that were modified from their default setting when using the function to carry out various analyses such as Shannon H' index, GAMs, Pearson correlations, linear regression on residuals, etc. For instance, "GAMs were fit using the function 'gam (y~s, method = "REML")' from the mgcv package v1.9-1 (Wood, 2017)." Furthermore, the interpretations and discussion rely heavily on Pearson correlations – please add some justification for why this method was chosen over others, i.e., Spearman rank-correlations. For datasets that follow a bivariate normal distribution, Pearson correlations are useful to measure linear relationships (not sure if you have tested for whether your datasets are normally distributed). However, if the datasets are nonnormally distributed or have relevant outliers, you might actually consider using an alternative like Spearman correlation to test for monotonic association. This could provide different interpretations, potentially stronger correlations, than what your current results indicate.

Results and Discussion

Lines 303 – 307: This section discussing the results of the cyanobacteria fraction of the data could be expanded a bit more. For instance, this potential dominance of *Prochlorococcus*

might align with the observed warming influence and advection of oligotrophic offshore waters into the study region as previously observed at the San Pedro Ocean Time-series (SPOT) where this was accompanied by a notable shift from cold-water ecotypes to warm-water ecotypes during 2014-2015 (Yeh and Fuhrman, 2022 - https://doi.org/10.1038/s41467-022-35551-4). Similarly, the 2015-2016 El Niño event also marked an increase in an open ocean ecotype of UCYN-A at SPOT (Fletcher-Hoppe et al., 2023 - https://doi.org/10.1038/s43705-023-00268-y) but it seems its presence and range of coverage was not detected with the cyanobacteria ASVs recovered from the samples collected in this study.

Figure 3: Consider specifying "All Cyanobacteria" on the figure's panel titles C and D to align with the description in the figure legend. And same thing for Figure S7.

Line 497: "...;however, contrary to predictions" Are there literary references to suggest that diversity and richness should be expected to be low in deep SCML samples – where/why did you have that hypothesis?

Figure 5: Are the samples highlighted in panel F only a subset of the samples from panel E? It is specified that the samples are ordered by the associated fucoxanthin concentrations, but it seems that only samples above a certain *dabA* expression threshold are included here – maybe clarify this selection criteria.

Technical comments:

Line 48: "Earth system**s** models" (make it as "system" – singular)

Line 137: Station 81.8 46.9 – are these two separate stations or just a unique nomenclature?

Line 481: "...for the mechanisms that underlying them." Awkward phrasing.

Line 535: Adjust to "...shown to produce DA and its production is..." You already introduced the acronym DA to represent domoic acid so you can maintain consistency this way.

Lines 542-544: Consider rephrasing the sentences to streamline the strucutre: "Dinoflagellates, including certain members in the genera *Alexandrium*, *Dinophysis*, and *Gonyaulax* and species *Gymnodinium catenatum* and *Lingulodinium polyedra*, may also cause HABs globally and in the region (Anderson et al., 2012, 2021; Trainer et al., 2010; Ternon et al., 2023)."

Lines 545 – 546: "although 39% of V4 and 55% of V9 18S copies..." Wouldn't referencing Figure S6B better point to these percentages – not sure the reference to Figure S13 here? Also, does blasting those sequences improve the taxonomic resolution to help better assess if there are potentially more HAB species which may currently be unassigned as HABs due to insufficient taxonomic resolution?